REMARKS

The current status of the claims is:

Claims 1-4, 10, 12, 14, and 18-21 are pending in the application. Claims 15-17 were canceled by previous amendment. Claims 5-9, 11, and 13 have apparently been withdrawn by the Examiner in accordance with MPEP 821 as being drawn to a non-elected species.

Claims 2-4, 8, 10, 12, 14, and 19 remain in their original form.

Claims 1, 18, and 20 are currently amended.

Claim 21 was previously presented.

The specification has been amended at paragraph 2 on page 14 to change "formulae (I)-(IV)" to "formulae (I)-(II)." As the specification only recites formulae (I) and (II) the reference to formulae (I)-(IV) is clearly a typographical error.

Claim 1 has been amended to replace "CNS disorder" to "CNS disorder associated with impaired prefrontal cortical function associated with activation of protein kinase C." Support for this amendment is found at least at paragraph 3 on page 4. Claim 1 has also been amended to replace "impaired cognitive performance" with "a working memory deficit." Support for this amendment is found at least on pages 6 – 7, in the figure captions for FIGURES 6 and 10. Claim 1 has also been amended to recite "administering orally or systemically" to a subject. Support for this amendment is found at least at page 15, paragraph 2. Claim 1 has also been amended to delete Formula II and to change C_1-C_6 alkyl to C_1-C_3alkyl in the definitions of R^4, R^5, R^6, R^7 and R^8 and to change "n is 0 to 5" to "n is 0 to 3." Support for this change is found in the application as filed at paragraph 6 which states that substituted alkyl groups containing 1-3 carbon atoms are preferred

Claim 18 has been amended to recite the chemical formula formerly given in Claim 15. This amendment was needed to make Claim 18 a proper independent claim. Support for this amendment is found in the application as filed, and at least in original Claim 15. The changes made to the formula recited in Claim 1 (deletion of Formula II, change C_1-C_6alky

Claim 21 has been amended to change "protecting a subject from developing a CNS disorder" to "for treating manic episodes in a bipolar patient." Support for this amendment can be found in the application as filed and at least at paragraphs 1 to 2 of page 2. Claim 21 has also been amended to change "a pharmaceutical composition according to claim 15" to "chelerythrine." Support for this amendment may be found at least at paragraph 3 of page 3.

Applicants thank Examiner Claytor and her supervisor Examiner Padmanabhan for the courtesy of a telephonic interview on October 31, 2007.

Claim Objections

The Examiner has objected to Claim 18-20 as being dependent on a canceled claim. Claims 18 and 20 have been rewritten in independent form and Claim 19 depends from Claim 18. Applicants respectfully request the Examiner withdraw the objection to Claims 18-20.

Rejections Under 35 USC §112

The Examiner has rejected claims 1-3, 10, 12, 14, and 18-21 under 35 USC §112, first paragraph. Applicants respectfully assert that the Claims as amended meet the requirements of 35 USC §112, first paragraph. Applicants understand the Examiner's rejection to be based on both the number of CNS disorders encompassed by the pending claims (all CNS disorders) and the number of compounds within the claim scope (all compounds of formulas I and II).

The Examiner contends that a skilled artisan would not be able to practice the invention without undue experimentation. In rejecting claims under 35 USC 112, first paragraph the test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue. *In re Angstadt*, 537 F.2d 498, 504, 190 U.S.P.Q. 214, 219 (CCPA 1976). The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *In re Certain Limited-Charge Cell Culture Microcarriers*, 221 USPQ 1165, 1174 (Int'l Trade Comm'n 1983), aff'd. sub nom., *Massachusetts Institute of Technology v. A.B. Fortia*, 774 F.2d 1104, 227 U.S.P.Q. 428 (Fed. Cir. 1985).

The Examiner has correctly cited *In re Wands* (858 F.2d. 731, 8 USPQ2d 1400 (Fed. Cir. 1988)) as setting forth the factors to consider when assessing whether a disclosure would have required undue experimentation. Applicants respectfully assert that no undue experimentation exists when the Wands factors are correctly applied to the amended claims. The Wands factors include (1) the nature of the invention, (2) the breadth of the claims, (3) the presence or absence of working examples (4) the amount of direction or guidance presented, (5) the quantity of experimentation necessary, (6) the state of the prior art, (7) the relative skill of those in the art, and (8) the predictability or unpredictability of the art.

In Wands, the Court held that the specification was enabling with respect to the claims at issue and found that even though considerable experimentation was needed to practice the claimed invention the amount of experimentation was not undue because "there was considerable direction and guidance" in the specification; there was "a high level of skill in the art at the time the application was filed;" and "all of the methods needed to practice the invention were well known." 858 F.2d at 740, 8 USPQ2d at 1406. The situation is quite similar here.

The claims as amended recite a method of treating a CNS disorder associated with impaired prefrontal cortical function associated with activation of protein kinase C or a working memory deficit. One of ordinary skill in the art of treating CNS disorders would readily understand the phrase "CNS disorder associated with impaired prefrontal cortical function associated with activation of protein kinase C" to include at least those disorders listed in the specification: bipolar disorder, major depressive disorder, schizophrenia, post-traumatic stress disorder, anxiety disorders, attention deficit hyperactivity disorder, and Alzheimer's disease (behavioral symptoms) as each of these disorders is associated with impaired prefrontal cortical function.

During the interview Applicants proposed the wording "treating a CNS disorder associated with impaired prefrontal cortical function" and Examiner Padmanabhan indicated that "treating a CNS disorder associated with impaired prefrontal cortical function associated with uncontrollable stress" would be acceptable due to the clear support for this phrase at paragraph 2, page 3. Applicants did not suggest the current wording during the interview but not that there is also clear support for "treating a CNS disorder associated with impaired prefrontal cortical function associated with activation of protein kinase C" at paragraph 3 on page 4 of the specification as filed. Applicants respectfully request this wording be entered as it is the most descriptive of brain function in patients with the claimed disorders, including bipolar disorder, which is the elected species.

The claimed methods now recite use of compounds of formula I, which formulas encompass chelerythrine and a number of close analogs of chelerythrine. The application provides a general method for preparing the claimed chelerythrine analogs at paragraph 4 of page 18 to page 19. One of ordinary skill would be able to make and use the claimed chelerythrine analogs to treat a CNS disorder associated with impaired prefrontal cortical function or a working memory deficit given the disclosure in the application.

During the interview the Examiner Padmanabhan stated he did not believe compounds of Formula II or compounds having large ether substituents at R_4 to R_8 were enabled for the claimed method. Applicants have amended Claim 1 to recite only compounds of Formula I and have amended the definitions of R_4 to R_8 to recite compounds having C_1-C_3 alkyl substituents rather than C_1-C_6 alkyl substituents. While it is Applicants' position that Claim 1 is enabled for all disclosed compounds these amendments are made to comply with the Examiners' suggestions and advance prosecution.

The application as filed provides considerable direction and guidance for using compounds of formula I for treating the disorders now claimed. The application provides chelerythrine as working example of a compound useful for treating a CNS disorder associated with impaired prefrontal cortical function or a working memory deficit. The application provides numerous examples of chelerythrine's

utility in treating CNS disorders associated with impaired prefrontal cortical function in rats and monkeys; e.g. Example 1 (data in FIGURE 8) demonstrates that chelerythrine blocks stress-induced cognitive deficits in rats and Example 2 (data in FIGURE 11) demonstrates that orally administered chelerythrine reverses the detrimental effects of stress on prefrontal cortical function. The specification provides data in Figures 6 and 10 indicating that chelerythrine can be used to block phenyleprine working memory deficits. One of ordinary skill would understand that chelerythrine and its close analogs can be used to treat working memory deficits.

The methods set forth in Examples 1 and 2 and elsewhere in the specification, which demonstrate chelerythrine's utility in the claimed method, can easily be applied to determine the utility of other compounds of formulas 1 and 2 in the claimed method.

The amount of experimentation is not undue. The application clearly demonstrates the utility of chelerythrine for treating a CNS disorder associated with impaired prefrontal cortical function or a working memory deficit and provides a number of methods useful for determining the disclosed chelerythrine analogs' utility in treating these disorders. *In vitro* methods are highly unreliable for determining the efficacy of compounds in treating psychiatric disorders. Whenever possible *in vivo* data is preferred. *In vivo* screening of compounds in rodent models of disease, such as the method given in Example 1 are routine in the art of central nervous system drug development. The amount of experimentation needed to determine which compounds of formulas 1 and 2 are most useful for treating CNS disorders associated with impaired prefrontal cortical function is a matter of only routine experimentation.

The Examiner has separately rejected Claim 20 under 35 USC §112, first paragraph for claiming "prevention." Applicants note that claims 20 as amended recites "treating manic episodes in a bipolar patient," rather than prevention generally. Applicants' position is "treating manic episodes in a bipolar patient" is enabled because it is well-established in the art that manic episodes in bipolar patients are correlated with prefrontal cortical dysfunction. See for example Sax, KW, et al., *Frontosubcortical Neuroanatomy and the Continuous Performance Test in Mania*, Am. J. Psychiatry (1999) 156: 139-141. Administration of compounds that inhibit protein kinase C is thought to improve prefrontal cortex function and thereby block (or prevent) the occurrence of manic episodes. The requirements of 35 USC §112, first paragraph are met for "preventing manic episodes in a bipolar patient." Applicants thank Examiner Padmanabhan for suggesting this language during the telephonic interview and for indicating that this language would be acceptable.

Applicants respectfully request the Examiner reconsider and withdraw the rejections under 35 USC §112, first paragraph.

Rejections Under 35 USC §103

The Examiner has rejected Claims 1-4, 10, and 18-21 as obvious over Aylward (US 2003/0195168) in view of Herbert (172(3) Biochem. Biophys. Res. Comm. 993-999 (1990)).

It is our position that the discussion set forth in AYLWARD in view of HERBERT does not make treating bipolar disorder with chelerythrine obvious because a person of ordinary skill would not have expected that such use of chelerythrine would be successful and thus the Examiner has made a *prima facie* case for obviousness[1].

A worker of ordinary skill would not have expected that systemically-administered chelerythrine would interact with protein kinase C in the brain. The blood brain barrier presents a major impediment to drug efficacy in treating CNS disorders, very often restricting drug access to critical sites in the brain. The large number of conferences and books and other publications on the subject demonstrates the difficulty of directing drugs across the blood brain barrier. See, for example, *The Blood-Brain Barrier: Biology and Research Protocols,* Nag, S., editor, Humana Press (2003), *Biology and Physiology of the Blood-Brain Barrier: Transport, Cellular Interactions, and Brain Pathologies,* Couraud, P-O. and Scherman, D., editors, *Cerebral Vascular Biology Symposium*, Kluwer Academic Pub. (1995 Paris), and *The Blood-Brain Barrier and Drug Delivery to the* CNS, Begley, D.J., et al., editors, Dekker (2000).

The smaller and more lipophilic a drug is, the more likely it is to be taken into the brain. See Wilkinson, G.R. *Pharmacokinetics The Dynamics of Drug Absorption, Distribution, and Elimination,* in Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th edition, Hardman, J.G. and Limbird, L.E., editors. McGraw Hill (2001). Chelerythrine is a fairly large molecule, molecular weight > 750 D. It is also is a quaternary salt, charged at pH 7.4, the pH of blood, with and thus not lipophilic. Chelerythrine would not be expected to cross the blood brain barrier when administered non-centrally. A worker of ordinary skill would not expect that chelerythrine could cross the blood brain barrier and thus would have no reasonable expectation of success that chelerythrine could be used to treat biopolar disorder.

Chelerythrine was available as a research tool at the time the present application was filed. The ICN Pharmaceuticals Biochemicals and Reagents catalog for 2000 lists chelerythrine chloride as product no. 158888, and describes it as a specific inhibitor of protein kinase C. Despite the availability of chelerythrine and its known effect on PKC researchers were not previously motivated to administer chelerythrine for the treatment of bipolar disorders. Instead researchers either injected chelerythrine directly into the brain of laboratory animals or used it *in vitro*, for example by applying chelerythrine to brain slices. See, for example Serano, P.A., et al., *Protein Kinase C Inhibitor Chelerythrine Disrupts Memory Function*, 109(2) Behav. Neurosci. 278-284 (1995) and Schulte, G. and Fredholm, B.B., *Diverse*

[1] A reasonable expectation of success is required for a *prima facie* case of obviousness based on choosing from a finite number of identified, predictable solutioerns. See Federal Register v. 72, no. 195, p. 57532.

Inhibitors of Intracellular Signaling Act as Adenosine Receptor Antagonists, 14(2) Cell Signal 109-113 (2002).

Longstanding use of chelerythrine to treat peripheral disorders, but not CNS disorders, also supports the conclusion that workers of ordinary skill did not expect that chelerythrine could be used to treat bipolar disorder at the time the application was filed. As early as 1971 chelerythrine was shown to be effective for the treatment of *Candida* infections in rodents when administered topically or injected. Vichkanova, S.A. and Adgina, V.V., *Chemotherapeutic Properties of Sanguirythrine in Treatment of Experimental Candidoses*, 18(10) Antibiotiki (Moscow) 902-905 (1973). Chelerythrine has also been administered orally to humans for the treatment of gingivitis (U.S. Patent No. 5,013,553). Other reports in the patent literature discuss the use of chelerythrine for treating varicose veins, particularly hemorrhoids (U.S. Patent No. 6,210,680), increasing tumor apoptosis (U.S. Patent No. 6,025,365), and inhibiting solid tumor growth (U.S. Patent No. 6,426,351). In each of these patents chelerythrine was used to treat peripheral, rather than CNS, disorders.

A review of the literature provides only two examples of chelerythrine being implicated in *in vivo* treatment of nervous system disorders prior to the filing date of the application. U.S. Patent No. 6,815,450 became publicly available just before the present application was filed and provides some insight into the understanding of the utility of chelerythrine among those skilled in the relevant art at the time the application was filed. The '450 patent discusses the use of chelerythrine and a number of other PKC inhibitors in regenerating growth of an adult mammalian CNS axons subject to growth inhibition (Col. 8, Table 2). The '450 patent discusses the response of CNS neurons in the spine to a direct infusion of a PKC inhibitor. The '450 inventors discuss how PKC inhibitors may be induced to cross the blood brain barrier by surgical methods, direct injection or infusion, intraocular implants, or within/ on implants (Col. 4, lines 7 – 15). There is no indication that the inventors believed that any of the disclosed PKC inhibitors, including chelerythrine, could be used for CNS treatment, without direct application, much less by oral administration.

The second patent reference, U.S. Patent No. 6,407,058, discusses the effect of PKC activators and inhibitors, including chelerythrine on the permeability of biological barriers.
The '058 patent discloses a model (Figure 1) which predicts activation of PKC increases permeability across tight junctions, such as the blood-brain barrier. Reciprocally, the '058 patent discloses that inhibition of PKC, e.g., by chelerythrine, will decrease tight junction permeability. (Col. 2, ll. 45-54). Therefore, one of ordinary skill in the art would understand the '058 patent to teach that treatment with chelerythrine REDUCES the permeability of blood-brain barrier. It is completely unexpected that chelerhythrine itself would then be able to cross the tightened blood-brain barrier.

It is also our opinion that the chelerythrine's activity in the brain is unexpected. A clinical study on the clinical effects of tamoxifen in patients suffering from acute mania also demonstrates that chelerythrine's activity in the brain is unexpected. Bebchuk, J. et al. *A Preliminary Investigation of a*

Protein Kinase C Inhibitor in the Treatment of Acute Mania, 57(1) Arch. Gen. Psych. 95-97 (2000). The authors of this study postulated that PKC inhibitors could be used to treat acute mania, and chose to test this hypothesis by administering high doses of tamoxifen[2]. In their report they state that tamoxifen was "the only relatively selective PKC inhibitor available for human use." *Id.* at 95. This statement was made despite the fact the chelerythrine had already been recognized as a PKC inhibitor and have been used for in humans for non-CNS conditions. See, for example U.S. Patent No. 5,013,553, discussed above. Authors of the tamoxifen study also noted that tamoxifen was known to cross the blood brain barrier. Authors of the tamoxifen study noted several problems with chronic use of tamoxifen to treat CNS conditions, including its long-term depressogenic properties. *Id.* Thus those of skill in the art, motivated to use PKC inhibitors to treat bipolar disorder, did not recognize that chelerythrine could be used for this purpose. Nor were they motivated to experiment with chelerythrine for treatment of mania, in spite of the recognized flaws in using tamoxifen for this purpose.

The importance and unexpected nature of Applicants' result is underscored by a recent publication in the field stating "There is currently only one relatively selective protein kinase C inhibitor that crosses the blood brain barrier – tamoxifen." Zarate, Jr. CA, et al. "*Efficacy of a protein kinase C inhibitor (tamoxifen) in the treatment of acute mania: a pilot study*," 9 Biopolar Disorders 261-270 (2007), attached hereto as Exhibit A. To this date workers of greater than ordinary skill in the relevant art do not reasonably expect that chelerythrine can be administered non-centrally to treat CNS disorders.

Clinically relevant methods of treating CNS disorders include oral and systemic administration of a therapeutic agent. During the interview the language "administered orally or systemically" was suggested. Applicants thank the Examiner for this suggestion. Applicants have adopted this language citing the numerous examples of systemic administration methods listed in the specification at page 15 paragraph 2.

It is Applicants position that at the time of the invention one of skill in the art would have had no reasonable expectation of success that chelerythrine or its close analogs could be given orally or systemically to treat a CNS disorder associated with impaired prefrontal cortical function associated with activation of protein kinase C or a working memory deficit and thus the invention is not obvious.

Applicants respectfully request the Examiner to reconsider and withdraw the rejection under 35 USC §103.

Rejoinder request

Rejoinder of the withdrawn claims 5-9, 11, and 13 is requested. Should the Examiner agree that the rejections under 35 USC 112, first paragraph and 35 USC 103(a) have been overcome for at least the use of chelerythrine for the treatment of, bipolar disorder, (the elected species) rejoinder and examination

[2] Patients received 60 mg tamoxifen/ day. Recommended doses of tamoxifen do not exceed 40 mg/ day. AstraZeneca prescribing information for Nolvadex® (tamoxifen citrate) revision 03/05.

of claimed directed to non-elected species is proper.

Consideration and allowance of these claims are respectfully requested. The foregoing is believed to be fully responsive to the action.

If there are any charges with respect to this amendment, or otherwise, please charge them to Deposit Account No. 06-1130 maintained by applicant's attorneys.

Respectfully submitted,
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Exhibit A

Bipolar Disorders 2007: 9: 561–570

BIPOLAR DISORDERS

Original Article

Efficacy of a protein kinase C inhibitor (tamoxifen) in the treatment of acute mania: a pilot study

Zarate Jr CA, Singh JB, Carlson PJ, Quiroz J, Jolkovsky L, Luckenbaugh DA, Manji HK. Efficacy of a protein kinase C inhibitor (tamoxifen) in the treatment of acute mania: a pilot study. Bipolar Disord 2007: 9: 561–570. © Blackwell Munksgaard, 2007

Objectives: Considerable preclinical biochemical and behavioral data suggest that protein kinase C inhibition would bring about antimanic effects. Notably, the structurally highly dissimilar antimanic agents lithium and valproate, when administered in therapeutically relevant paradigms, attenuate protein kinase C inhibition function. There is currently only one relatively selective protein kinase C inhibitor that crosses the blood–brain barrier available for human use – tamoxifen. Our group recently conducted a single-blind study with tamoxifen in acute mania and found that it significantly decreased manic symptoms within a *short period* of time (3–7 days). In this study, we investigated whether antimanic effects can be achieved with a protein kinase C inhibitor in subjects with mania.

Methods: In a double-blind, placebo-controlled study, 16 subjects with bipolar disorder, manic or mixed, with or without psychotic features, were randomly assigned to receive tamoxifen (20–140 mg/day; n = 8) or placebo (n = 8) for three weeks. Primary efficacy was assessed by the Young Mania Rating Scale.

Results: Subjects on tamoxifen showed significant improvement in mania compared to placebo as early as five days, an effect that remained significant throughout the three-week trial. The effect size for the drug difference was very large (d = 1.08, 95% confidence interval 0.45–1.71) after three weeks (p = 0.001). At study endpoint, response rates were 63% for tamoxifen and 13% for placebo (p = 0.12).

Conclusions: Antimanic effects resulted from a protein kinase C inhibitor; onset occurred within five days. Large, controlled studies with selective protein kinase C inhibitors in acute mania are warranted.

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Key words: antimanic – mania – protein kinase C – tamoxifen

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Bipolar disorder (BD) is a common, chronic recurrent mood disorder that is among the most disabling of all medical illnesses and affects the lives of millions worldwide. Although there have been substantial gains in the treatment of acute mania with lithium, valproate, carbamazepine and atypical antipsychotic drugs, many patients fail to respond adequately or to tolerate these treatments (1–3). Furthermore, except for lithium, all available Food and Drug Administration-approved treatments for BD fall in the category of anticonvulsant or antipsychotic drugs and were developed first for some other indication (4). Indeed, it is striking that more than 50 years since the identification of the antimanic effects of lithium, we have yet to develop a new treatment specifically for BD. There is no doubt that the development of novel therapeutics has been greatly hampered by the fact

None of the co-authors of this study have any possible conflict of interest, financial or otherwise.

that our understanding of *the precise molecular and cellular underpinnings* of this complex disorder is in its infancy.

In the absence of knowledge about the precise pathophysiology of the illness, a number of laboratories have focused upon elucidating the therapeutically relevant targets of our most effective existing medications in an effort to develop improved therapeutics. In this context, although a number of acute, *in vitro* effects of antimanic agents have previously been identified, their therapeutic effects in the treatment of BD are seen only after chronic administration (5). In addition, chemically distinct mood stabilizers such as lithium and valproate, which have fairly distinct primary biochemical targets, show similar (albeit clearly not identical) clinical antimanic effects. When coupled, these observations suggest that downstream, adaptive changes in cell function, rather than acute actions on direct pharmacological targets, may be of greater importance. Since medications like antidepressants or antimanics regulate multiple targets upon chronic administration, several laboratories (6, 7) have developed a set of criteria that must be met in order to increase the likelihood that the biochemical target is therapeutically relevant. These include: (i) validation at the protein and functional level; (ii) observation with structurally highly dissimilar but clinically efficacious agents (e.g., lithium and valproate); (iii) occurrence at a dose/plasma level consistent with clinical therapeutic effects; (iv) occurrence in a timeframe consistent with clinical therapeutic effects; (v) localization to brain regions implicated in the disorder; (vi) when possible, relevance to known illness pathophysiology. It is noteworthy that several independent laboratories (8–12) have shown that the protein kinase C (PKC) signaling cascade meets all these criteria and may, therefore, represent a novel direct biochemical target for the treatment of mania. Importantly, a whole genome association study of BD has recently been completed; the gene demonstrating by far the strongest association with BD was diacylglycerol kinase, an immediate regulator of PKC (vide infra) (13).

The protein kinase C signaling cascade

Protein kinase C represents a family of enzymes highly enriched in brain, where it plays a major role in regulating both pre- and post-synaptic aspects of neurotransmission. It is among the major intracellular mediators of signals generated upon external stimulation of cells via a variety of neurotransmitter receptors (including muscarinic m_1 and m_3, noradrenergic α_1, serotonergic $5HT_{2A}$, metabotropic glutamatergic receptors, etc.), which induce the hydrolysis of various membrane phospholipids. Protein kinase C exists as a family of closely related subspecies, has a heterogeneous distribution in brain (with particularly high levels in presynaptic nerve terminals), and plays a crucial role in the regulation of neuronal excitability, neurotransmitter release, regulation of synaptic plasticity and various forms of learning and memory. A considerable amount of biochemical data support the potential involvement of PKC and its substrates in bipolar patients and changes in PKC signaling pathways after treatment with lithium or valproate (7, 9, 14–17).

Both lithium and valproate, when administered in therapeutically relevant paradigms, bring about isozyme-specific decreases in PKC α and ϵ (7, 9, 18). Additional studies demonstrated that chronic lithium and valproate also regulate the expression of the most prominent PKC substrate [myristoylated alanine-rich protein kinase C substrate (MARCKS)] in brain, a protein that has been implicated in signaling and neuroplastic events associated with cytoskeletal remodeling (19, 20).

In humans, Friedman and colleagues (15) investigated PKC activity and PKC translocation in response to serotonin in platelets obtained from patients with BD before and during lithium treatment. They reported that the ratios of platelet membrane-bound to cytosolic PKC activities were elevated in subjects in a manic episode. In addition, serotonin-elicited platelet PKC translocation was found to be enhanced in those subjects. In brain tissue, Wang and Friedman (21) measured PKC isozyme levels, activity and translocation in postmortem brain tissue from patients with BD. They reported increased PKC activity and translocation in the brains of patients with BD compared to controls, effects which were accompanied by elevated levels of selected PKC isozymes in cortices of the patients.

In animal models of mania, several studies have demonstrated that both acute and chronic amphetamine use produce an alteration in PKC activity, its relative cytosol to membrane distribution, as well as the phosphorylation of a major PKC substrate, GAP-43, which has been implicated in long-term alterations of neurotransmitter release (22, 23). Furthermore, PKC inhibitors have been shown to block the acute responses to amphetamine (10) and cocaine (as assessed by both behavioral and *in vivo* microdialysis studies), as well as cocaine-induced sensitization (11, 24). Increased hedonistic drive and increased tendency to abuse drugs are well known facets of manic behavior; notably, PKC inhibitors attenuate these important

facets of the manic-like syndrome in rodents (25–30). Importantly, recent preclinical studies have specifically investigated the antimanic effects of tamoxifen *per se* (since this is the only CNS-penetrant PKC inhibitor available for humans). These studies showed that tamoxifen significantly reduced amphetamine-induced hyperactivity and risk-taking behavior (25). Finally with respect to cognitive dysfunction associated with mania, Birnbaum and associates (26) have demonstrated that excessive activation of PKC dramatically impaired the cognitive functions of the prefrontal cortex, and that inhibition of PKC protected cognitive function. These data suggest that PKC may play an important role in some of the cognitive features of mania. In summary, preclinical biochemical and behavioral data support the notion that PKC activation may result in manic-like behaviors, whereas PKC inhibition may be antimanic.

PKC inhibitors: novel agents for the treatment of acute mania?

As demonstrated above, the PKC signaling pathway is clearly a target for the actions of two structurally dissimilar antimanic agents – lithium and valproate. Do these effects of lithium and valproate on PKC signaling actually have any clinical relevance? As discussed, the PKC signaling pathway fulfils most of the criteria for therapeutic relevance. The fact that opposite effects are observed with psychostimulants (pro-manic agents), the rodent behavioral data and the modest available human data all suggest that the inhibition of PKC may be therapeutically relevant in the treatment of acute mania. Thus, there is a clear need to investigate the potential efficacy of a direct PKC inhibitor in the treatment of acute mania.

A clear limitation has been the fact that there is only one relatively selective PKC inhibitor available for human use that crosses the blood–brain barrier – tamoxifen. Tamoxifen, a synthetic non-steroidal anti-estrogen, has been widely used in the treatment of breast cancer (27). A number of its anticancer effects are due to estrogen receptor antagonism (27), but it has become clear in recent years that it is also a potent and selective PKC inhibitor at therapeutically relevant concentrations (28, 29). Tamoxifen clearly crosses the blood–brain barrier, has efficacy in the treatment of malignant gliomas, and is fairly well tolerated even at high doses (up to 200 mg/day) (30). Tamoxifen, among the least toxic of anticancer agents, is the most widely used hormonal therapy for breast cancer, and has been approved as a chemopreventive agent in women at high risk for breast cancer.

In an earlier single-blind study (31), we found that tamoxifen 20–80 mg/day for 4–15 days (8.4 ± 4.2 days) resulted in a significant decrease in manic symptoms rated by the Young Mania Rating Scale (YMRS) (32) within 3–7 days in seven patients with DSM-IV bipolar I disorder (BD I), current episode manic or mixed.

Based on the preclinical data and our preliminary clinical study, we postulated that PKC inhibition would bring about antimanic effects. Therefore, the objective of the present double-blind trial was to determine if the relatively selective PKC inhibitor tamoxifen exerts antimanic effects in patients with BD.

Methods

Patient selection

Subjects were recruited from referrals from local inpatient psychiatric units in the DC Washington Metropolitan Area. Men and women, aged 18–65 years, who were inpatients with a current diagnosis of BD, current episode manic or mixed with or without psychotic features as diagnosed by means of the Structured Clinical Interview for Axis I DSM-IV Disorders-Patient Version (SCID-P) (33) were eligible to participate. Because of the unknown consequences of the anti-estrogen effects of tamoxifen on uterine tissue at the doses studied, the first eight subjects randomized included only men. With the safety data on the first eight subjects, the protocol was amended to include women who were premenopausal with regular menstrual cycles. All subjects were studied at the National Institute of Mental Health (NIMH) Clinical Research Center in Bethesda, MD, between March 2003 and August 2006. Subjects were required to have a score of ≥14 on the YMRS (32) at screening and at randomization (baseline) and not to have >20% improvement in YMRS total scores between the screen and randomization visits. In addition, subjects were required to have been previously treated with at least one of the following drugs at some time during the course of their bipolar illness: lithium, valproate, carbamazepine, or an atypical (except for clozapine) or typical antipsychotic. This criterion was included for ethical reasons, so that treatment-naïve subjects were not enrolled in the study.

All subjects were in good physical health as determined by medical history, physical exam, blood labs, electrocardiogram, chest X-ray, urinalysis and toxicology. Subjects were free of

comorbid substance abuse or dependence for at least one month and judged clinically not to be a serious suicide risk. Other exclusion criteria included: QT_C >450 ms at screening (high doses of tamoxifen have been reported to cause prolongation in QT_C); participation in a clinical trial of another investigational drug within one month prior to screening; presence of a coagulation disorder; history of deep venous thrombosis or pulmonary embolism; presence of retinal pathology; or current clinically significant abnormal laboratory tests. Comorbid Axis I anxiety disorder diagnoses were permitted if they did not require current treatment. Final selection was made by consensus of the investigation team.

The study was approved by the NIMH Institutional Review Board. All subjects provided written informed consent before entry into the study. Informed consents and ongoing study participation were monitored by the Central Office for Recruitment and Evaluation (CORE) at NIMH.

The initial study plan included provisions for 20 participants per group. Given the present robust change in YMRS on tamoxifen, at least a moderate effect size (d = 0.50) would be achieved, even with no overall response in the remaining sample.

Study design

This single-site, three-week, randomized, double-blind, parallel group inpatient study compared tamoxifen and placebo in the short-term treatment of BD I. Before randomization to the three-week double-blind treatment phase, subjects underwent a 2–7-day screening period. All psychotropic medications, with the exception of benzodiazepines, were discontinued at least 48 h before randomization. Subjects who met enrollment criteria were randomized to tamoxifen or placebo in a 1:1 ratio. Patients were randomized using a computer-generated algorithm generated by the NIMH pharmacy. All subjects and research staff were blinded to randomization codes. Study treatments were supplied in identical 20-mg capsules containing either placebo or tamoxifen. Tamoxifen was purchased from AstraZeneca Pharmaceuticals, Wilmington, DE, USA.

Patients received flexible dosing of either tamoxifen (20–140 mg/day) or placebo. A maximal dose of 140 mg/day of tamoxifen was chosen to determine whether any additional benefit is achieved by using doses higher than we used in our open-label study (up to 80 mg/day) (31). Doses as high as 200 mg/day of tamoxifen have been reported to be well tolerated (30). The starting dose of tamoxifen was 20 mg/day. After the first day of therapy, the daily dose could be adjusted upward or downward, as clinically indicated, by 20-mg increments or decrements within the allowed dose range of 20–140 mg/day. The dose of tamoxifen was increased by 20 mg/day until one of the following endpoints was reached: (i) response criterion (defined as a 50% decrease in YMRS ratings from baseline); (ii) intolerable side effects; or (iii) the maximum allowable dose of tamoxifen had been reached (140 mg/day). Dose reductions were permitted in cases of adverse events. The minimal dose of tamoxifen allowable during the study was 20 mg/day.

Concomitant use of lorazepam was allowed during the double-blind therapy at up to 2 mg/day for the first 10 days. Lorazepam was not permitted beyond the initial 10 days after randomization.

Assessment and outcome measures

Subjects were rated on a daily basis for the first 7 days and then weekly thereafter (on days 14 and 21). The YMRS was the primary outcome measure and the secondary outcome measures were the Montgomery-Asberg Depression Rating Scale (MADRS) (34) and the Positive and Negative Syndrome Scale (PANSS) (35).

The primary efficacy variable, as defined by the protocol, was the reduction from baseline of the YMRS total score after three weeks of therapy. Raters (research nurses, a physician and psychologist), who trained together to establish reliability, performed patient ratings. High inter-rater reliability for the SCID [intraclass correlation coefficient (ICC) = 0.88], YMRS (ICC = 0.91), PANSS (ICC = 0.87) and MADRS (ICC = 0.81) were obtained. Clinical response was defined as a 50% or greater decrease in the YMRS score from baseline.

All adverse events were recorded with the NIMH Data Safety Event Codes. Because tamoxifen has been reported to prolong the QT_C interval in humans (36) and higher doses of tamoxifen (up to 140 mg/day) were to be utilized in this study, daily electrocardiograms (ECGs) were obtained. A decision was made to withdraw subjects who, in the blinded phase, had a QT_C of >480 ms.

Statistical analyses

The *a priori* hypothesis was that overall manic symptoms would change with tamoxifen treatment, so the primary outcome measure was the total YMRS score. The difference between tamoxifen

and placebo from baseline to 21 days for the intent-to-treat sample was examined using a fixed-effects linear mixed model with restricted maximum likelihood estimation. According to Akaike's Information Criterion and Schwarz's Bayesian Criterion, a first-order autoregressive covariance structure appeared to be the best fitting variance-covariance matrix. Significant effects were examined with Bonferroni *post hoc* tests. Additional outcome measures were examined in a secondary analysis to understand potential changes in depression (MADRS), psychosis (PANSS subscales), QT_C, and individual manic symptoms (YMRS items). The size of effect for the tamoxifen–placebo difference is Cohen's d with corresponding 95% confidence interval (CI). Since some patients were taking lorazepam during the trial, the use of lorazepam was compared for the drug groups over time and the YMRS and MADRS analyses were run again with lorazepam dose as a time-dependent covariate.

Fisher's exact test was used to compare the treatment groups on the proportion of responders and remitters at endpoint. Responders had an improvement of at least 50% in YMRS scores from baseline to endpoint and remitters had a YMRS score of 7 or less at endpoint (37).

Significance was evaluated at $p \leq 0.05$, two-tailed. Means and associated standard deviations are reported. As all participants who received a baseline rating also had at least one rating on a drug, all available data were included in the analysis. SPSS 14.0.2 was used for this analysis.

Results

Patients

A total of 58 subjects were screened, yielding 16 subjects for randomization who met DSM-IV TR criteria for BD, current episode manic or mixed with or without psychotic features. A total of 42 subjects were excluded as they did not meet entry criteria (n = 24), refused to participate (n = 9), had unstable medical illness (n = 7), or for other reasons (n = 2) (Fig. 1). Eight subjects received tamoxifen and eight received placebo. Completion rates (i.e., ratings obtained through week 3) were comparable for both drug groups [tamoxifen: four of eight (50%); placebo: five of eight (63%)] (Fisher's exact test, p = 1.00).

The subjects' demographic and clinical characteristics are summarized in Table 1. There were 14 males and 2 females, with a mean age of 35.4 ± 7.8 years. A total of 56% had a lifetime diagnosis of any substance abuse or dependence. Based on DSM-IV criteria using the SCID-P, 69% of patients had a manic index episode, and 50% were experiencing psychosis. The mean length of illness was 16.4 ± 4.9 years, the mean duration of the

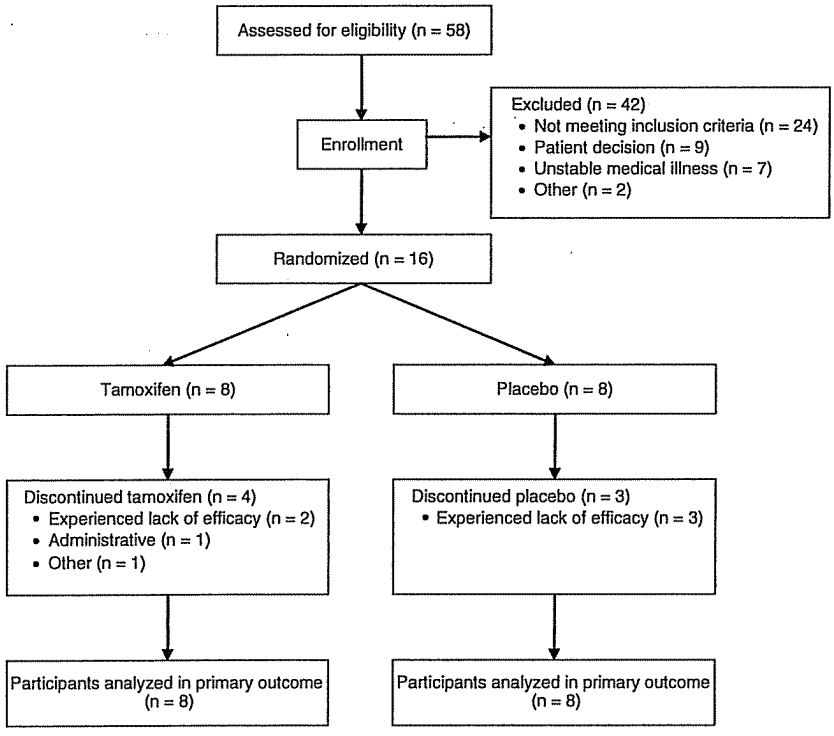

Fig. 1. Enrollment, randomization, withdrawals and completion of the two treatment phases (n = 16).

Table 1. Patient and illness characteristics

Characteristic	Tamoxifen (n = 8) Mean (SD)	Placebo (n = 8) Mean (SD)	p-value[a]
Age, years	36.1 (10.0)	34.6 (5.5)	0.72
Current episode, days	35.9 (37.5)	32.0 (17.9)	0.80
Length of illness	16.5 (2.8)	16.4 (6.6)	0.96
No. of previous hospitalizations, lifetime	4.5 (6.1)	3.3 (3.7)	0.63
No. of previous episodes of mania, lifetime	6.0 (6.2)	4.8 (2.9)	0.61
No. of episodes of mania, previous 12 months	1.3 (1.0)	1.0 (0.76)	0.59
No. of previous episodes of depression, lifetime	3.6 (3.0)	3.4 (3.0)	0.87
No. of episodes of depression, previous 12 months	0.75 (0.70)	0.38 (0.74)	0.32
YMRS total	30.3 (7.0)	24.3 (5.3)	0.08
MADRS total	20.1 (10.9)	12.3 (4.7)	0.08
PANSS total	82.1 (24.1)	57.5 (17.3)	0.04
PANSS positive	24.1 (6.2)	19.8 (7.0)	0.22
PANSS negative	16.0 (9.3)	9.3 (1.4)	0.06
	n (%)	n (%)	p-value[a]
Male	8 (100)	6 (75)	0.47
White	7 (88)	4 (50)	0.28
Psychotic	5 (63)	3 (38)	0.62
Current episode mixed state	3 (38)	2 (25)	1.00
Rapid cycler[b]	4 (50)	2 (25)	0.61
Lifetime diagnosis of substance abuse	5 (64)	4 (50)	1.00
Previous medication use			
Lithium	8 (100)	8 (100)	1.00
Valproate	6 (75)	7 (88)	1.00
Carbamazepine	3 (38)	4 (50)	1.00
Olanzapine	4 (50)	5 (63)	1.00
Risperidone	1 (13)	4 (50)	0.28
Ziprasidone	1 (13)	2 (25)	1.00
Quetiapine	3 (38)	2 (25)	1.00
Aripiprazole	0 (0)	2 (25)	0.47
Typical antipsychotic	2 (25)	4 (50)	0.61
Patients treated with any of the above three medications	8 (100)	8 (100)	1.00

[a]Treatment difference, tamoxifen versus placebo; derived from analysis of variance for continuous measures and from Fisher's exact test for categorical measures.
[b]Rapid cycler defined as ≥4 mood episodes in the preceding 12 months based on DSM-IV criteria.
SD = standard deviation; YMRS = Young Mania Rating Scale; MADRS = Montgomery-Asberg Depression Rating Scale; PANSS = Positive and Negative Syndrome Scale.

current manic episode was 33.9 ± 28.4 days, and the mean numbers of lifetime episodes of mania and depression were 5.4 ± 4.7 and 3.5 ± 2.9, respectively. Except for PANSS total scores, there were no statistically significant differences in any demographic or illness characteristics between treatment groups. Even doubling the sample size for each group would not create a statistical difference between groups on demographics. Historical illness characteristics and previous medication use are presented in Table 1.

Lorazepam was used during the trials of four of eight (50%) tamoxifen patients and six of eight (75%) placebo patients. A linear mixed model examining lorazepam use over time by drug group showed a significant main effect for drug ($F = 14.30$, $df = 1,18.3$, $p = 0.001$), but no time effect ($F = 0.57$, $df = 8,82.5$, $p = 0.80$) or interaction with time ($F = 1.08$, $df = 8,82.5$, $p = 0.38$). The placebo group (mean 0.804 ± 0.534) used more lorazepam over the course of the trial than the tamoxifen group (mean 0.114 ± 0.564). The doses for the groups were different at baseline and remained at approximately the same levels over the course of the trial.

Efficacy

The linear mixed model for the YMRS showed a significant interaction between time and drug ($F = 2.68$, $df = 8,70.0$, $p = 0.01$), but the main effects for time ($F = 1.68$, $df = 8,70.0$, $p = 0.12$) and drug ($F = 0.92$, $df = 1,11.6$, $p = 0.36$) were not significant. Figure 2 shows the estimated marginal means following restricted maximum likelihood estimation from the linear mixed model.

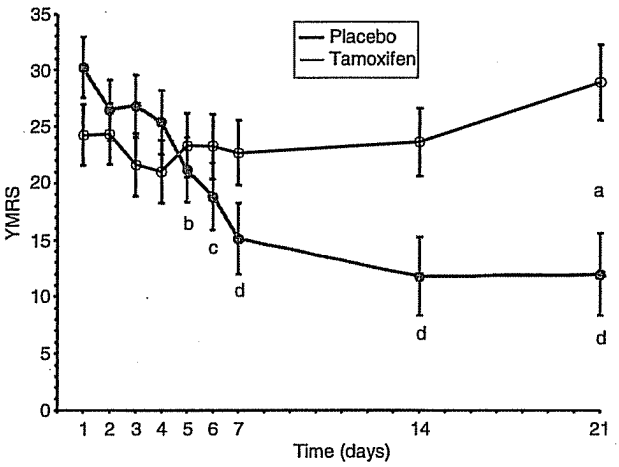

Fig. 2. Change in scores on the Young Mania Rating Scale (YMRS) over three weeks (n = 16). Tamoxifen versus placebo: $^{a}p < 0.05$ after correction. Week versus baseline for tamoxifen: $^{b}p < 0.05$; $^{c}p < 0.01$; $^{d}p < 0.001$ after correction.

Following Bonferroni correction, *post hoc* tests show a significant difference between placebo and tamoxifen at 21 days (d = 1.08, 95% CI 0.45–1.71). The tamoxifen group showed significant improvement from baseline at day 5 (d = 0.59, 95% CI 0.17–1.00), continuing through day 21 (d = 1.11, 95% CI 0.59–1.64); the placebo group showed no significant change from baseline at any point. For the YMRS, the mean change on placebo at 3 weeks was 4.7 ± 4.1 and the change on tamoxifen was −18.3 ± 4.3. Therefore, tamoxifen appears to decrease manic symptoms. Using lorazepam dose as a time-dependent covariate did not alter these effects (drug × time: F = 2.57, df = 8,70.5, p = 0.02).

On tamoxifen, five of eight (63%) patients had 50% or greater improvement in YMRS scores at the endpoint of the study, while one of eight (13%) had similar improvement on placebo (Fisher's exact p = 0.12). In addition, two patients on tamoxifen and none on placebo achieved a YMRS score ≤7 (Fisher's exact p = 0.47).

Additional models were run in the same manner as the total YMRS for the individual items to identify specific areas where tamoxifen may be helpful to patients. Significant time × drug interactions were found for elevated mood (F = 2.58, df = 8,61.3, p = 0.02), increased motor activity or energy (F = 3.45, df = 8,67.6, p = 0.002), increased sexual interest (F = 2.28, df = 8,78.8, p = 0.03), and appearance (F = 2.20, df = 8,71.8, p = 0.04). For elevated mood, scores on placebo were significantly higher than on tamoxifen from days 5–21 and baseline scores were significantly higher than those for days 5 and 7–21 on tamoxifen. With increased motor activity or energy, placebo was significantly higher than tamoxifen on days 6 and 21 and scores were significantly lower than baseline on tamoxifen at days 5–21. Sexual interest was significantly higher at baseline than on day 2 of tamoxifen. In addition, appearance was significantly worse on placebo compared to tamoxifen at day 2. Significant main effects for drug were found for decreased sleep (F = 9.24, df = 1,23.8, p = 0.006) and increased speech (F = 9.72, df = 1,19.8, p = 0.005); values on placebo were higher than for tamoxifen. Although interactions were not significant for these symptoms, the groups were not different at baseline, suggesting that these symptoms decreased over time on tamoxifen. Beyond the significant effects, the mean score for each symptom was lower on tamoxifen than placebo at day 21 and lower at day 21 compared to baseline on tamoxifen.

The linear mixed model evaluating depression (MADRS) scores showed significant main effects for time (F = 3.85, df = 8,73.5, p = 0.001) and drug (F = 6.42, df = 1,15.1, p = 0.02), but no interaction (F = 1.08, df = 8,73.5, p = 0.39). Since the tamoxifen group appeared to have higher baseline MADRS scores, an additional analysis was run in which the baseline score was used as a covariate. This analysis indicated no significant drug effect (F = 1.44, df = 1,17.6, p = 0.25) or interaction (F = 1.22, df = 7,61.1, p = 0.31), suggesting that tamoxifen does not increase depression significantly. Lorazepam dose did not change the MADRS results (drug × time: F = 1.08, df = 8,73.0, p = 0.39).

Pearson correlations between the changes in YMRS and MADRS scores at endpoint were not significant for placebo or tamoxifen, regardless of whether absolute change (placebo: r = 0.25, p = 0.55; tamoxifen: r = 0.07, p = 0.87) or percent change (placebo: r = 0.36, p = 0.38; tamoxifen: r = 0.28, p = 0.50) was used. Thus, improvement in mania was not significantly associated with worsening or improvement in depressive symptoms.

The PANSS was divided into three separate scores (positive, negative and general symptoms) before analysis. For positive and general symptoms, there were no drug effects (PANSS positive: drug: F = 1.06, df = 1,15.5, p = 0.32; drug × time: F = 0.99, df = 8,67.3, p = 0.45; PANSS general: drug: F = 4.48, df = 1,14.7, p = 0.052; drug × time: F = 1.45, df = 8,62.9, p = 0.19). For negative symptoms, there was a significant effect for drug (F = 8.55, df = 1,19.4, p = 0.009), but no interaction (F = 0.38, df = 8,59.5, p = 0.93). Since the tamoxifen group

Table 2. Treatment-emergent adverse events that first occurred or worsened in severity during double-blind therapy

	Tamoxifen	Placebo	p-value[a]
Decreased appetite	6 (75)	1 (13)	0.04
Headache	3 (38)	5 (63)	0.62
Fatigue	3 (38)	4 (50)	1.00
Depression	3 (38)	2 (25)	1.00
Tension	3 (38)	1 (13)	0.57
Weight loss	3 (38)	0 (0)	0.20
Gastrointestinal distress	2 (25)	4 (50)	0.61
Concentration problems	2 (25)	3 (38)	1.00
Dry mouth	2 (25)	1 (13)	1.00
Dizziness	2 (25)	1 (13)	1.00
Nausea	1 (13)	3 (38)	0.57
Increased dreaming	1 (13)	3 (38)	0.57
Taste abnormality	1 (13)	1 (13)	1.00
Constipation	1 (13)	1 (13)	1.00
Increased salivation	1 (13)	0 (0)	1.00
Nasal congestion	1 (13)	0 (0)	1.00
Loose stools	1 (13)	0 (0)	1.00
Flatulence	1 (13)	0 (0)	1.00
Palpitations	1 (13)	0 (0)	1.00
Hot flashes	1 (13)	0 (0)	1.00
Suicidal ideation	1 (13)	0 (0)	1.00
Pain muscle/joint/bone	1 (13)	0 (0)	1.00
Irritability	0 (0)	3 (38)	0.20
Diarrhea	0 (0)	2 (25)	0.47
Dry skin/irritation	0 (0)	2 (25)	0.47
Weight gain	0 (0)	1 (13)	1.00

[a]Frequencies were analyzed using the Fisher's exact test.

appeared to have higher scores at baseline, the analysis was run again with baseline as a covariate. There were no significant drug effects after controlling for baseline.

Adverse effects

Overall, tamoxifen was well tolerated. No patient discontinued treatment because of an adverse event and no serious adverse events were observed. The only treatment-emergent event with a statistically significant more frequent occurrence in the tamoxifen group compared to the placebo group was loss of appetite ($p = 0.03$, Fisher's exact test) (Table 2). A linear mixed model with QT_C interval data showed a significant interaction between drug and time ($F = 2.12$, $df = 8,70.7$, $p = 0.04$), but *post hoc* tests showed no significant drug differences at any time-point or change from baseline. The closest point to significant was an increase from baseline on tamoxifen at 21 days ($p = 0.054$, uncorrected). No subject withdrew due to a QT_C >480 ms.

Discussion

In this double-blind, placebo-controlled pilot trial, the relatively selective PKC inhibitor tamoxifen was associated with significant antimanic effects in individuals with BD. Our hypothesis was that directly inhibiting PKC would result in antimanic effects, and that these effects would be seen more rapidly than previously shown with lithium or valproate (which exert their primary effects considerably upstream of PKC, and ultimately regulate PKC through a cascade of events). Indeed, the antimanic effects of tamoxifen were rapid, showing significant improvement as early as day 5. While brain tamoxifen levels are unavailable, this timeframe is consonant with achieving sufficiently high tamoxifen levels with the dosing strategy used (increases of 20 mg/day). Tamoxifen demonstrated an improvement of −18.3 points from baseline to endpoint on the YMRS and achieved statistical significance compared to placebo, which demonstrated a worsening of 4.7 points. These findings support the results of our single-blind study with tamoxifen in acute mania (31) and that of other recent studies with tamoxifen (38, 39).

A limitation of the study – which was unavoidable given the current status of development of central nervous system-penetrant PKC inhibitors for human use – is the fact that tamoxifen is not entirely PKC-selective and has anti-estrogen effects. Thus, despite the strong *a priori* theoretical grounds for postulating that a PKC inhibitor would have antimanic effects, we clearly cannot rule out the potential contributory effects of estrogen receptor blockade.

It is noteworthy that there are reports describing improvement in manic symptoms in women with the anti-estrogen agent danazol (40), as well as estrogen and progesterone agents (38, 41); however, this potential confound awaits the availability of a completely selective, safe PKC inhibitor that crosses the blood-brain barrier. It could also be argued that the antimanic effect seen with tamoxifen was due to the use of lorazepam, which also has 'antimanic' effects. However, covarying for lorazepam dose did not affect the significance of the findings with tamoxifen.

In terms of side effects, tamoxifen was generally well tolerated over the short-term. The only side effect that was found to be more common with tamoxifen than with placebo was loss of appetite. Anorexia has been previously reported to occur with tamoxifen and it has been speculated to be the result of the accumulation of malonyl-COA in the hypothalamus and inhibition of fatty acid synthase expression specifically in the ventromedial nucleus of the hypothalamus (42). Contrary to previous reports that tamoxifen might be depressogenic (43, 44), we found no significant changes in depression ratings in either our previous (31) or the present

study. Linear mixed models of MADRS total scores in the current trial failed to show a significant worsening of depressive symptoms with tamoxifen.

These preliminary results clearly need to be interpreted with caution. First, the group size was small and, as such, the results should be considered tentative and pilot, but sufficiently positive to pursue larger controlled trials with PKC inhibitors in mania. Second, our results may not be generalizable to patients with certain characteristics (e.g., presence of substance use disorders). Third, these results may not apply beyond the acute treatment phase of bipolar mania.

In conclusion, the study findings support our hypothesis that PKC inhibition would bring about antimanic effects in patients with BD. The findings of this pilot study suggest that PKC inhibition might be relevant to the antimanic effects of lithium and valproate. Large, controlled studies with selective PKC inhibitors in acute bipolar mania are warranted.

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